REMARKS

Reconsideration of this application is respectfully requested.

Claim 32 was objected to under 37 CFR § 1.75(c) as being in improper form because a multiple dependent claim should refer to other claims in the alternatives only. Claim 32 has been cancelled. Thus, this objection may be withdrawn.

Claim 33 was objected to under 37 CFR § 1.75(c) as being in improper form because a multiple dependent claim should refer to other claims in the alternatives only. Claim 33 has been amended to depend from claims 28 "or" 40. Thus, this objection may also be withdrawn.

Claim 32 was rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter, which applicant regards as the invention. As claim 32 has been cancelled, this ground for rejection may be withdrawn.

Claims 28-31 and 33 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Stanton et al (USPGPUB NO. US2001/0034023 published Oct. 25, 2001) in view of Larsen et al (Human mutation, 1999, 13,318-327). Claims 28 and 29 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Maekawa et al (Biochem. biophys. res. comm. 1996, 223, 520-525, cited in IDS filed May 9, 2005) in view of Larsen et al (Human mutation, 1999, 13, 318-327). Finally, Claims 28 and 32 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Stanton et al. (USPGPUB NO. US2001/0034023 published Oct. 25, 2001) in view of Larsen et al. (Human mutation, 1999, 13,318-327), and further in view of Gelfand et al. (USPN 5,487,902 issued Jul. 30, 1996).

Each of these grounds for rejection is respectfully traversed and reconsideration is requested for the following reasons.

The present invention is directed to the field of editing of ribonucleic acid (RNA) of the 5-HT2c receptor (5-HT2c-R). Editing of ribonucleic acid (RNA) can be considered as a major mechanism of control in post-transcriptional regulation of gene expression. It allows cells to produce multiple RNA molecules from a unique RNA transcript. The A to I conversion in mRNA sequence, catalysed by editing enzymes (ADARs), has been shown to be particularly active in brain and to play a strong putative role in the control of serotoninergic transmission via the 5-HT2c receptor. The existence of five potentially edited sites on the sequence coding for the intracellular peptidic loop of the protein leads to the expression of 32 possible mRNA isoforms, and subsequently, 24 possible isoforms of the receptor protein with different capacity of functional coupling. Consequently, a fast and accurate method to identify and determine the relative proportions of these mRNA isoforms is of primary importance.

Before the present invention was made, two methods had been used for this purpose. The first, based upon primer extension in the presence of an adequate dideoxynucleotide, made it possible to estimate the proportion of a given site, but could not provide the proportions of the different isoforms in which this site is edited (see enclosed article by *Niswender et al.*, 1999). The second, involving sequencing of a large number of complementary DNA clones of the 5-HT2c-R, is time-consuming, expensive, and far from able to obtain the homogeneity of relative errors, which are necessary to a convenient statistical analysis of a group of brain samples (see enclosed article by *Sohdi et al.* 2005).

The present invention (see amended claim 28 below) claims the use of a SSCP-CE method, which is a convenient basic process for discriminating the edited isoforms of the 5-HT.2c-R mRNA expressed in a given tissue extract after a single assay from a total RNA sample.

> 28. (amended) An SSCP method for obtaining, under given analytical conditions, the editing profile of the 5-HT.2c-R mRNA, using a specific tissue sample or using a sample of a population of eukaryotic cells, characterized in that it comprises the following steps:
>
> a) extraction of the total RNAs of said sample, followed, where appropriate, by purification of the mRNAs;
>
> b) reverse transcription of the RNAs extracted in step a) and synthesis of the double-stranded DNA;
>
> c) PCR amplification of the DNAs obtained in step b) using the following pair of primers specific for said mRNA which may be edited:
>
> PCR9 TGTCCCTAGCCATTGCTGATATGCT (SEQ ID No. 36); and
>
> PCR10 GCAATCTTCATGATGGCCTTAGTCCG (SEQ ID No. 37).
>
> d) where appropriate, purification of the PCR products obtained in step c);
>
> e) where appropriate, quantification of the PCR products obtained in step d);
>
> f) dissociation of the double-stranded DNAs to single-stranded DNAs, in particular by heating followed by abrupt cooling;
>
> g) separation of the single-stranded DNAs by capillary electrophoresis; and
>
> h) obtaining of the editing profile by reading of the fluorescence and, where appropriate, acquisition of the profile data by means of the exploitation system associated with the fluorescence reader.

The strategy used in the present invention for obtaining the mixture of cDNA involves, first, reverse transcription (RT) (step b), followed by PCR (step c). The reverse primer used for the RT can hybridize, for example, to the poly A tail of the extracted RNA obtained in step a).

The PCR of step c) is achieved by the use of a sense primer hybridizing in exon IV and a reverse primer hybridizing in exon V, giving a 250 bp-long fragment without any risk of amplifying genomic DNA, thus avoiding overestimation of the non-edited form. In these conditions, assuming that the efficiency of the RT is identical for the putative 32 different mRNA forms generated by A to I editing, the second PCR of step c) should maintain the initial relative proportions of the different forms, since amplification was carried out to its final point in an identical way for all different forms present in a unique mixture.

Therefore, the ultimate quantification takes account of the initial percentages of the different mRNA forms. This basic formulation of the assay was designed in order

-To exclude the risk of amplification of genomic DNA;

-To be efficient from small tissue samples (total RNA ≥50ng have been shown to give linear and reproducible results); and

-To discriminate by capillary electrophoresis (CE) the 32 isoforms of the mRNA (the editing sites are in an arrow of 13 nucleotides!) after SSCP of the RT/PCR products.

As indicated in pararagraph [0125] of the present application,

> [0125] Preferably, in the SSCP method according to the invention, the pair of primers used in step c) is chosen such that the PCR products obtained are at least 100 bases in length, more preferably at least 125, 150, 175, 200, 225 or

> 250 bases in length, in order to allow folding characteristic of the editing form of each or of the two strands separated after step g)".

As also indicated in paragraph [0235] and [0236] of the present application,

> [0235] The pair of primers PCR9, PCR10, used for the PCR amplification of a fragment of the 5-HT.sub.2c-R gene, was chosen in DNA regions that are identical in humans, mice and rats.

(This could be important to have a consensus SSCP-CE method for use in mouse and rat, which are often used in the pharmaceutical field, particularly for screening therapeutic compounds).

> [0236] The resulting PCR amplification product is 250 base pairs in size, in the 3 species human, mouse and rat.

This has been achieved after a large set of experiments to choose the best length of strands. The CE conditions, the adequate fluorescent probes, and the adequate design of the CE signal can be optimized by the skilled person.

An example of the capacity of the process (which is within claim 28) has been published in 2007 (Poyau et al., Elecrophoresis 2007, 28, 2843-2852, see copy enclosed). The pair of primers

PCR9 TGTCCCTAGCCATTGCTGATATGCT; (SEQ ID No. 36) and

PCR10 GCAATCTTCATGATGGCCTTAGTCCG. (SEQ ID No. 37)

of the present invention are those used in the Poyau et al. publication in the first PCR of the nested PCR. This has been considered as a completely new method for solving the difficult analytical problem of the quantification of the complex distribution of 5-HT2c-R mRNA isoforms in tissue samples.

Regarding the cited prior art, Larsen et al. only describes the use of the SSCP-CE for identifying allelic variant, i.e the presence of one point mutation in a fragment

compared to the wild type fragment. In general, in the case of allelic variant identification, one or at most two different cDNA fragments are present in the sample mixture and have to be separated (one if homozygote for that mutation, two if heterozygote). In the case of the 5-HT.2c-R mRNA, 32 isoforms of the cDNA fragment (all the editing sites are in an arrow of 13 nucleotides!) can be potentially present in the sample mixture and have to be separated.

None of the cited documents Larsen et al., Stanton et al, or Maekawa et al., discloses or suggests that at least 13 different isoforms (see Figures 1C to 1O) of the present application can be separated, identified, and quantified from the same mixture.

These deficiencies are not remedied by Gelfand et al. The cited Gelfand et al. reference only provides guidance in the choosing of a pair primers to improve PCR in a general way. The choice of primers having a length comprised between 15-30 nucleotides is well known by the skilled person for implementing the general method of PCR amplification.

In summary, the problem that is solved by the present invention is to provide a fast and accurate method able to identify and to quantify each of the 5-HT.2c-R RNA isoforms (32 possible), which can be potentially present at the same time in a total RNA extract sample from a mammal, such as a human, rat, or mouse. The principal difficulty confronting the worker in the art is that the five editing sites (responsible for the 32 potential different isoforms) are in an arrow of 13 nucleotides. For the first time (see the publication Poyau et al. which has been accepted for this reason by the referees), the inventors have demonstrated that it is possible to identify and to quantify all these potential isoforms in the same sample (possible from human, rat, or mouse with the

same pair of primers) by a direct method (SSCP-CE), provided that the PCR product chosen is about 250 bp and that the primers chosen are those identified in Claim 28). None of the cited prior art discloses or suggests that a direct SSCP followed by CE method with a PCR product chosen of 250 bp could separate, identify, and quantify the presence of these potential isoforms in a same sample.

Accordingly, the rejection under 35 U.S.C. § 103 may be withdrawn.

Please grant any additional extensions of time required to enter this response and charge any additional required fees to our Deposit Account 06-0916.

Respectfully submitted,

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